

AMENDMENTS TO THE SPECIFICATION

At page 8, please replace the third and fourth full paragraphs as follows:

Figure 3 illustrates the DNA sequence of PCNA. (SEQ ID NO: 59).

Figure 4 illustrates the translated protein sequence of PCNA. (SEQ ID NO: 60).

At page 9, replace the third full paragraph as follows:

Figure 8 illustrates the DNA sequence of genomic RFC clones. (SEQ ID NO: 61).

Genomic sequences encoding the P38 and P55 subunits are located in tandem, respectively. The sequence encoding P38 contains an intein. As used herein, the term "intein" includes, but is not limited to protein splicing elements. These elements are involved in the post-translational processing of pre-proteins. The coding regions of the P38 and P55 subunits are bracketed []. The intein sequence is enclosed in parentheses ().

Please replace the paragraph bridging pages 9 and 10 as follows:

Figure 9 illustrates the translated protein sequence of the genomic RFC clone. (SEQ ID NO: 62). The sequence encoding P38 and P55, respectively, are enclosed in parentheses (), while the sequence of the intein is bracketed []. The * indicates a stop codon.

At page 10, please replace the first and second full paragraphs as follows:

Figure 10 illustrates the translated protein sequence of recombinant P55 clone.

(SEQ ID NO: 63).

Figure 11 illustrates the translated protein sequence of recombinant P38 clone.

(SEQ ID NO: 64).

At page 11, please replace the first and second full paragraphs as follows:

Figure 16 illustrates a cDNA sequence of a clone expressing RFA. (SEQ ID NO:

65).

Figure 17 illustrates the translated protein sequence of RFA. (SEQ ID NO: 66). The theoretical molecular weight is 41.3 kDa. The native protein may start at the third methionine.

At page 12, please replace the first through the twelfth full paragraphs as follows:

Figure 22 illustrates the DNA sequence of recombinant helicase 2. (SEQ ID NO:
67). This helicase has demonstrated PCR enhancing activity.

Figure 23 illustrates the DNA sequence of recombinant helicase 3. (SEQ ID NO:
68).

Figure 24 illustrates the DNA sequence of recombinant helicase 4. (SEQ ID NO:
69).

Figure 25 illustrates the DNA sequence of recombinant helicase 5. (SEQ ID NO:
70).

- Figure 26 illustrates the DNA sequence of recombinant helicase 6. (SEQ ID NO: 71).
- Figure 27 illustrates the DNA sequence of recombinant helicase 7. (SEQ ID NO: 72).
- Figure 28 illustrates the DNA sequence of recombinant helicase dna2. (SEQ ID NO: 73). This helicase has demonstrated PCR enhancing activity.
- Figure 29 illustrates the translated protein sequence for helicase 2. (SEQ ID NO: 74). The theoretical molecular weight is 87.9 kDa + 4.0 kDa (CBP affinity tag).
- Figure 30 illustrates the translated protein sequence for helicase 3. (SEQ ID NO: 75). The theoretical molecular weight is 100.0 kDa + 4.0 kDa.
- Figure 31 illustrates the translated protein sequence for helicase 4. (SEQ ID NO: 76). The theoretical molecular weight is 105.0 kDa + 4.0 kDa.
- Figure 32 illustrates the translated protein sequence for helicase 5. (SEQ ID NO: 77). The theoretical molecular weight is 86.8 kDa + 4.0 kDa.
- Figure 33 illustrates the translated protein sequence for helicase 6 + 4.0 kDa (CBP affinity tag). (SEQ ID NO: 78).

Please replace the paragraph bridging pages 11 and 12 as follows:

- Figure 34 illustrates the translated protein sequence for helicase 7. (SEQ ID NO: 79). The theoretical molecular weight is 126.0 kDa + 4.0 kDa.

At page 13, please replace the first full paragraph as follows:

Figure 35 illustrates the translated protein sequence for helicase dna2 + 4.0 kDa (CBP affinity tag). (SEQ ID NO: 80).

At page 14, please replace the third and fourth full paragraphs as follows:

Figure 40 illustrates the DNA sequence of recombinant helicase 8. (SEQ ID NO: 81). Molecular weight is 82.6 kD + 4 kDa CBP tag.

Figure 41 illustrates the translated protein sequence of recombinant helicase 8. (SEQ ID NO: 82).

Please replace the table bridging pages 29 and 30 as follows:

Table 1

Gene Name	Forward Primer	Reverse Primer
RFC P98/P38	GACGACGACA <u>AAGATGAGCGA</u> AGAGATTAGAGAA (SEQ ID NO: 2)	<u>GGAACAAGACCCGTTCACTTCT</u> TCCAATTAGGGT (SEQ ID NO: 3)
RFC P55	GACGACGACA <u>AAGATGCCAGA</u> GCTTCCCTGGGT (SEQ ID NO: 4)	<u>GGAACAAGACCCGTTCACTTT</u> TAAGAAAGTCAA (SEQ ID NO: 5)
PCNA	GACGACGACA <u>AAGATGCCATT</u> CGAAATAGTCTTG (SEQ ID NO: 6)	<u>GGAACAAGACCCGTTCACTCCT</u> CAACCCTGGGGCTA (SEQ ID NO: 7)
RFC P98 Intein Deletion Primers*	ACTACAGCGGCTTG (SEQ ID NO: 8)	CTTCCGACACCAGGG (SEQ ID NO: 9)
RFA	GACGACGACA <u>AAGATGATCAT</u> GAGTGCATTACAAAAGAAGA AATAATC (SEQ ID NO: 10)	<u>GGAACAAGACCCGTTCACATCA</u> CCCCCAATTCTTCCAATTCCC (SEQ ID NO: 11)
dna2 helicase	GACGACGACA <u>AAGATGAACAT</u> AAAGAGCTTCATAAACAGGCT T (SEQ ID NO: 12)	<u>GGAACAAGACCCGTTCAAATGC</u> TATCCTCGTTAGCACAACATA (SEQ ID NO: 13)

Helicase 2	<u>GACGACGACAAGATGATTGA</u> GGAGCTGTTCAAGGGATTAG AGAGTGAAAT (SEQ ID NO: 14)	<u>GGAACAAGACCCGTTCATCTTT</u> TTACGGCAAATGCGAATTCTTC TCCCTT (SEQ ID NO: 15)
Helicase 3	<u>GACGACGACAAGATGTTAAT</u> AGTTGTAAGACCAGGAAGAA AAAAGAATGA (SEQ ID NO: 16)	<u>GGAACAAGACCCGTTCATCGTC</u> TCTCACCCCTCAAAATTTCCT TCTTC (SEQ ID NO: 17)
Helicase 4	<u>GACGACGACAAGATGCACAT</u> ATTGATAAAAAAAGGCAATAAA AGAGAGATT (SEQ ID NO: 18)	<u>GGAACAAGACCCGTCTATTCCC</u> AAAACTTCTAGTTGGATGTAG TGTTC (SEQ ID NO: 19)
Helicase 5	<u>GACGACGACAAGATGTTATTAA</u> AGGAGAGACTTAATACAGCC TAGGATAT (SEQ ID NO: 20)	<u>GGAACAAGACCCGTCTACTCCT</u> CATCCTCTATATATGGGGCAGT TATTAA (SEQ ID NO: 21)
Helicase 6	<u>GACGACGACAAGATGCTCAT</u> GAGGCCAGTGAGGCTAATGA TAGCTGATG (SEQ ID NO: 22)	<u>GGAACAAGACCCGTCTAGCTTA</u> ACTTAAGTAAATGCCTATCTTC TTCT (SEQ ID NO: 23)
Helicase 7	<u>GACGACGACAAGATGATCGA</u> AGGTTACGAAATTAAACTAGC TGTTGTAAC (SEQ ID NO: 24)	<u>GGAACAAGACCCGTTCAAAAAC</u> <u>CTTCCCAGGTATGCGGGGGTC</u> GCT (SEQ ID NO: 25)
Helicase 8	<u>GACGACGACAAGATGAGGGT</u> TGATGAGCTGAGAGTTGATG AGAGGATA (SEQ ID NO: 26)	<u>GGAACAAGACCCGTTCAAGATT</u> TGAGAAAGTAATCAAGGGTACT TTTCT (SEQ ID NO: 27)

At page 40, please replace the first full paragraph as follows:

Probe was generated from a 200 bp PCR product amplified from *Methanococcus jannaschii* genomic DNA using the following primers:

Oligo # 576: GAT GAA AGA GGG ATA GAT (SEQ ID NO: 36)

Oligo # 577: ATC TCC AGT TAG ACA GCT (SEQ ID NO: 37)

These PCR primers were designed to anneal to regions flanking a 200 bp sequence of the *Methanococcus jannaschii* RF-C gene that exhibits 52% amino acid identity to the RF-C gene from human. (See Section 2 under Results below).

At page 44, please replace the first paragraph, after the subheading “A. Primer Extension Assay.,” as follows:

The *Pyrococcus furiosus* accessory proteins were tested for their ability to stimulate the processivity of cloned Pfu polymerase activity on primed single-stranded M13 DNA. One version of this assay provided for detecting extension products under non-denaturing conditions using ethidium bromide staining. For this assay, a reaction cocktail was made containing:

5 µg/ml	single-stranded M13 mp 18(+) strand DNA (Pharmacia cat# 27-1546-01)
275 ng/ml	40-mer primer (5' GGT TTT CCC AGT CAC GAC GTT GTA AAA CGA CGG CCA GTG C 3') (<u>SEQ ID NO: 38</u>)
200 µM	each dNTP
1X	cPfu buffer water to 20 µl.

Single stranded M13 DNA was mixed with primer, buffer, and water. The mix was heated to 95°C for 2 minutes and then cooled to room temperature. The rest of the reaction components were added. Each 20 µl reaction contained 0.05 units of cloned Pfu polymerase and varying amounts of PCNA and RFC. For assessing *P. furiosus* RF-C enhancement, assays contained 0.025 µl of *P. furiosus* PCNA (about 1 ng), and varying amounts of native *P. furiosus* RF-C. The reactions were incubated at 72°C for 15 minutes. 2 µl DNA loading dye (50% glycerol, 1xTBE, .05% bromphenol blue + .05% xylene cyanol) was added to each sample and 15 µl of sample with dye was loaded in each well of a 1% agarose gel (Reliant, FMC cat# 54907). The gel was stained with ethidium bromide. The double-stranded M13 can be seen as a brightly staining product that migrates higher than a 12 kb marker similar to the position where a

double-stranded M13 DNA control migrates. In this assay, one looks for an increase in the size of products synthesized when PCNA or PCNA + RFC are added to Pfu. Ethidium bromide staining is proportional to the amount of double-stranded DNA produced from primed single-stranded M13.

Please replace the paragraph bridging pages 47 and 48 as follows:

The assay cocktail contains:

10 µg/ml	single-stranded M13 mp 18 (+) strand DNA (Pharmacia cat# 27-1546-01)
100 ng/ml	40-mer primer (GGT TTT CCC AGT CAC GAC GTT GTA AAA CGA CGG CCA GTG C) (<u>SEQ ID NO: 39</u>)
1X	cPfu buffer
200 mM	KCl
30 µM each	dATP, dCTP, dGTP
3 µM	dTTP
5 µM	³ H-dTTP (NEN cat# NET-221H) (100 µCi/mL)
100 U/ml	cloned Pfu polymerase

Recombinant accessory factors or fractions derived from native *P. furiosus* are assayed for their ability to restore polymerase activity to the above cocktail. 1 µl samples were added to 10 µl of reaction cocktail, and reactions were incubated at 72°C for 30 minutes. Reactions were spotted onto DE81 filter papers, which were then washed and counted as described above.

At page 49, please replace the first full paragraph under the heading "D. Gel Shift Assay," as follows:

A 38 base oligo (5' GGT TTT CCC AGT CAC GAC GTT GTA AAA CGA CGG CCA GT 3') (SEQ ID NO: 40) was incubated with RFA samples at 95°C for 10 minutes,

followed by 72°C for 2 minutes, prior to loading on a 4-20% acrylamide gradient gel (Novex) in 1x TBE buffer. Bands were visualized by SYBER green staining (Molecular Probes) and UV illumination. DNA binding activity is monitored by looking for a retardation in the migration of the oligo (higher band) in the presence of RFA. Single-strand DNA binding activity is verified by showing a shift in band position using a single-stranded oligo but no shift using a double-stranded DNA duplex.

Please replace the paragraph bridging pages 50 and 51 as follows:

PCR reactions were carried out under standard conditions. In general, amplification reactions (50 µl) contained 200-450 µM each dNTP, 1x PCR buffer, 50-200 ng of human genomic DNA template (or 100 ng Stratagene's Big Blue transgenic mouse genomic DNA for the 0.5 kb target), 100 ng of each primer, and 2.5-5U of TaqPlus® Long DNA polymerase blend, PfuTurbo DNA polymerase, or Taq2000 DNA (Stratagene) polymerase. TaqPlus® Long PCRs were carried out in 1x buffer including: 50 mM Tricine pH 9.0, 8 mM ammonium sulfate, 0.1% Tween-20, 2.3 mM MgCl₂, and 75 ng/ml BSA. PCRs using PfuTurbo or Taq2000 DNA polymerase were carried out with the PCR buffers provided with the enzymes (Stratagene). Reactions were cycled in 200 µl thin-walled tubes using any of the following temperature cyclers: Stratagene RoboCycler® 96 temperature cycler fitted with a hot top assembly, Perkin Elmer GeneAmp PCR System 9600, or MJ Research PTC-200 Peltier thermocycler. The sequences of the PCR primers are given below:

23kb β-globin

Forward primer: 5' CAC.AAG.GGC.TAC.TGG.TTG.CCG.ATT 3'

Reverse primer: 5'-AGC.TTC.CCA.ACG.TGA.TCG.CCT.TTC.TCC.CAT-3'

Forward primer: 5'-CAC AAG GGC TAC TGG TTG CCG ATT-3' (SEQ ID NO: 44)

Reverse primer: 5'-AGC TTC CCA ACG TGA TCG CCT TTC TCC CAT-3' (SEQ ID NO: 45)

30kb β-globin

Forward primer: 5'-CTC.AGA.TAT.GGC.CAA.AGA.TCT.ATA.CAC.ACC-3'

Reverse primer: 5'-AGC.TTC.CCA.ACG.TGA.TCG.CCT.TTC.TCC.CAT-3'

Forward primer: 5'-CTC AGA TAT GGC CAA AGA TCT ATA CAC ACC-3' (SEQ ID NO: 46)

Reverse primer: 5'-AGC TTC CCA ACG TGA TCG CCT TTC TCC CAT-3' (SEQ ID NO: 47)

2.1 kb Alpha 1 Anti-Trypsin

Forward primer: 5'-GAG.GAG.AGC.AGG.AAA.GGT.GGA.AC-3'

Reverse primer: 5'-GAA.AAT.AGG.AGC.TCA.GCT.GCA.G-3'

Forward primer: 5'-GAG GAG AGC AGG AAA GGT GGA AC-3' (SEQ ID NO: 48)

Reverse primer: 5'-GAA AAT AGG AGC TCA GCT GCA G-3' (SEQ ID NO: 49)

5.2 kb Alpha 1 Anti-Trypsin

Forward primer: 5'-GAG.GAG.AGC.AGG.AAA.GGT.GGA.AC-3'

Reverse primer: 5'-GCT.GGG.AGA.AGA.CTT.CAC.TGG-3'

Forward primer: 5'-GAG.GAG.AGC.AGG.AAA.GGT.GGA.AC-3' (SEQ ID NO: 50)

Reverse primer: 5'-GCT.GGG.AGA.AGA.CTT.CAC.TGG-3' (SEQ ID NO: 51)

0.5kb λlacI (transgenic mouse genomic DNA)

lambda primer: 5'-GAC.AGT.CAC.TCC.GGC.CCG-3'

lacZ primer: 5'-CGA.CGA.CTC.GTG.GAG.CCC-3'

lambda primer: 5' GAC AGT CAC TCC GGC CCG-3' (SEQ ID NO: 52)

lacZ primer: 5' CGA CGA CTC GTG GAG CCC-3' (SEQ ID NO: 53)

Please replace the paragraph bridging pages 52 and 53 as follows:

P. furiosus PCNA was first identified in column fractions produced during fractionating native *P. furiosus* extracts. PCNA was co-purified with Pfu DNA polymerase during the Q and SP column procedures discussed above. Peak PCNA activity could be resolved from peak DNA polymerase activity using the heparin sepharose column, but all PCNA-containing fractions were contaminated with DNA

polymerase activity. To isolate native PCNA, fractions that could restore DNA polymerase activity to salt-inactivated Pfu DNA polymerase were studied. Such "restoration" activity was detected in column fractions eluting off the Heparin sepharose (Figure 1). An active column fraction was then subject to SDS-PAGE and gel slices were excised and extracted to remove proteins. DNA polymerase activity was found in a gel slice recovered from a position in the gel corresponding to the migration of proteins between 64-98 kDa. In contrast, PCNA activity was recovered from a gel slice that was located at a position lying between the 30 and 36 kDa protein markers (Figure 2). A protein band, migrating at 35 kDa, was visible on SDS-PAGE gels. This protein was transferred to a PVDF membrane (Bio Rad) and sent for amino terminal sequencing. The N-terminal sequence of the 35 kDa protein was:

PFEIVFEGA**K**EFAQ**I**LDTASKL(H,I)DEAAFKV**T**EDG--MR (SEQ ID NO: 54) (where (H,I) means either amino acid could be present, and - means that any amino acid could be present). A BLAST search of DNA sequence databases identified the 35 kDa protein as exhibiting significant homology to known eukaryotic PCNA sequences.

At page 57, please replace the second full paragraph as follows:

A 200 bp sequence encompassing the region encoding the 67-amino acid region was amplified from *Methanococcus jannaschii* genomic DNA using the following primers: 5' GAT GAA AGA GGG ATA GAT (SEQ ID NO: 36) and 5' ATC TCC AGT TAG ACA GCT (SEQ ID NO: 37). The *Methanococcus jannaschii* sequence was used to probe a *P. furiosus* genomic DNA library.